AD

EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4050

PROPHYLAXIS AGAINST SARIN POISONING IN THE RAT BY ORAL ADMINISTRATION OF 2-PAMCI

by

Peter Zvirblis
Albert A. Kondritzer

November 1966

Medical Research Laboratory
Research Laboratories
US ARMY EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010

EDGEWOOD ARSENAL TECHNICAL REPORT

EATR 4050

PROPHYLAXIS AGAINST SARIN POISONING IN THE RAT BY ORAL ADMINISTRATION OF 2-PAMCl

by

Peter Zvirblis
Albert A. Kondritzer

Physiology Department

Project 1C622401A097
Task 1C622401A09709

Medical Research Laboratory
Research Laboratories
US ARMY EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010

FOREWORD

The work described in this report was authorized under Project 1C622401A097, Medical Defense Aspects of Chemical Agents (U), and Task 1C622401A09709, Lethal Agents (U). This work was started in November 1959 and was completed in February 1960.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

Acknowledgment

Gratitude is expressed to Dr. John C. Atkinson, Biostatistics Office, and his staff for the statistical evaluation of the data in this report.

DIGEST

The aims of the present study were to (a) establish the parameters associated with oxime concentration in plasma following oral administration of 2-PAMCl (2-pyridine aldoxime methochloride) to rats, (b) determine the combined effect of prophylaxis with 2-PAMCl and treatment with atropine on the toxicity of sarin (isopropyl methylphosphonofluoridate, GB) in rats, and (c) establish a correlation between oxime level in plasma and protection afforded against sarin poisoning.

It was concluded that:

1. When 2-PAMCl is administered orally to rats, the plasma oxime level rises rapidly, reaches a maximum after about 1 hr, and then decreases at a first-order rate. The biological half-life of 2-PAMCl in the rat is estimated to be 1.4 hr.

2. A prophylactic effect of 2-PAMCl, when used in conjunction with atropine, has been demonstrated in rats poisoned with sarin.

3. The protection afforded by 2-PAMCl against sarin poisoning in rats can be correlated with the oxime level in the plasma at the time of sarin poisoning.

CONTENTS

		Page
I.	INTRODUCTION	7
II.	METHODS	7
	A. Experimental Animals	7
	B. Oral Administration of 2-PAMCl	7
	C. Determination of Oxime in Plasma	8
	D. Sarin-Toxicity Experiments	8
III.	RESULTS	8
	A. Oxime Concentration in Plasma	8
	B. Prophylaxis of Sarin Poisoning With 2-PAMCl	9
IV.	DISCUSSION	9
V.	CONCLUSIONS	9
	LITERATURE CITED	11
	APPENDIXES	13
	A. Tables A-I and A-II	15
	B. Figures B-1 and B-2	17
	DISTRIBUTION LIST	19
	DD FORM 1473 (DOCUMENT CONTROL DATA - R&D)	25

PROPHYLAXIS AGAINST SARIN POISONING IN THE RAT BY ORAL ADMINISTRATION OF 2-PAMCl

I. INTRODUCTION.

Askew[1] demonstrated prophylaxis against sarin (isopropyl methylphosphonofluoridate, GB) poisoning in the rat by the intraperitoneal administration of 100 mg/kg of 2-PAMI (2-pyridine aldoxime methiodide) and the subcutaneous administration of 17 mg/kg of atropine sulfate 15 min before intoxication. When the same regimen was used therapeutically 30 sec after intoxication, the toxicity of the sarin was not diminished. Crook and coworkers[2] showed that oral administration of 2-PAM lactate or P_2S (2-PAM methanesulfonate) before poisoning and intramuscular administration of atropine after poisoning decreased the toxicity of inhaled sarin vapor in dogs.

For some drugs, the therapeutic response appears to be related to the blood level of the drug, and the elimination of many drugs (based on blood-level data) can be expressed as a first-order rate.[3] The biological half-life, $(t_{1/2})_b$, can be used to predict the blood level of the drug and, in many cases, the therapeutic response over a time course following administration.

The aims of the present study were to (a) establish the parameters associated with oxime concentration in plasma following oral administration of 2-PAMCl (2-pyridine aldoxime methochloride) to rats, (b) determine the combined effect of prophylaxis with 2-PAMCl and treatment with atropine on the toxicity of sarin in rats, and (c) establish a correlation between oxime level in plasma and protection afforded against sarin poisoning.

II. METHODS.

A. Experimental Animals.

The experimental animals in this study were female white rats weighing 200 to 250 gm. The rats were fasted for 24 hr before the experiment, but water was available at all times.

B. Oral Administration of 2-PAMCl.

Aqueous solutions of 2-PAMCl were prepared at suitable concentrations to permit a volume of 10 ml/kg for the dosages given (30 or 60 mg/kg). The tip of a 3-in., No. 13 stainless-steel hypodermic needle was reworked into an oval-shaped nodule with a diameter of 3.5 mm at its thickest part. This tube was attached to a 5-ml glass syringe. The animal's jaws were kept open

by means of a hard rubber bar with a hole through it; the tube was passed through this hole and (via the esophagus) into the stomach, where the 2-PAMCl solution was deposited.

C. Determination of Oxime in Plasma.

Blood samples were obtained at various times (seven to nine rats for each sampling) by heart puncture after light chloroform anesthesia. Samples of about 6 ml were collected in heparin-treated tubes and centrifuged; 2.0 ml of plasma were removed for analysis of 2-PAMCl by the ultraviolet method described by May and coworkers.[4] Appropriate plasma samples were obtained from control animals to determine the blank.

D. Sarin-Toxicity Experiments.

2-PAMCl was administered orally to rats by the method described in section II, B. After a predetermined time interval (1 or 2.5 hr), the animals were given a solution of sarin in 0.9% saline (0.5 ml/kg) subcutaneously, followed immediately by an intramuscular injection of atropine sulfate solution (0.5 ml/kg), which provided a dose of 17 mg/kg of body weight. The logarithmic doses of sarin were equally spaced, and three or four of the doses in each group gave partial mortality responses. Ten animals were used at each point. By the same procedure, the toxicity of sarin in control animals treated with atropine but not with 2-PAMCl was determined concurrently with that in animals pretreated with 2-PAMCl. The data were subjected to probit analysis for comparisons of the various groups.

III. RESULTS.

A. Oxime Concentration in Plasma.

Following oral administration of 2-PAMCl to rats, the oxime concentration in plasma rises rapidly and reaches a maximum after about 1 hr. The oxime concentration then decreases at a rate that can be approximated to be first order. Table A-I, appendix A, lists the mean values of oxime found in the plasma at various time intervals following oral administration of 2-PAMCl. (Both tables, A-I and A-II, are in appendix A.) In figure B-1, appendix B, the first-order plots were fitted by the method of least squares; the estimate of the biological half-life was 1.2 and 1.5 hr in the groups receiving 30 and 60 mg/kg of 2-PAMCl, respectively. (Both figures, B-1 and B-2, are in appendix B.)

B. Prophylaxis of Sarin Poisoning With 2-PAMCl.

The observed data for the prophylactic efficacy of 2-PAMCl against sarin poisoning in rats (table A-II) is expressed in terms of the potency ratio. *

When a log-log plot of the data is constructed (figure B-2), a good correlation is demonstrated between the oxime concentration in plasma and the increase in the LD50 of sarin, even though the doses of 2-PAMCl and the times before the sarin poisoning varied.

IV. DISCUSSION.

After oral ingestion of 30 or 60 mg/kg of 2-PAMCl by the rat and man,[5, **] (a) the maximum oxime concentration in plasma is reached more quickly in rats (about 1 hr) than in man (2 to 3 hr), (b) the maximum oxime concentration in plasma is about the same in both species, and (c) the biological half-life of 2-PAMCl is shorter in the rat (1.2 to 1.5 hr) than in man (about 2 hr). These observations would suggest that both absorption from the gut and the elimination of 2-PAMCl are slower in man than in the rat.

All comparisons in this study have been made at the LD50 level of sarin in the rat. The potency ratios may differ at other levels of toxicity. The data do not warrant any statement concerning statistically significant differences in the slopes of the dose-mortality curves, even though table A-II indicates consistently steeper slopes for the control groups when compared with the oxime-treated animals.

The control animals in these experiments were treated with atropine. Estimates of potency ratios based on controls receiving no atropine can be made by multiplying the potency ratio by 1.4, the factor by which 17 mg/kg of atropine increases the LD50 of sarin in rats.[1]

V. CONCLUSIONS.

It was concluded that:

1. When 2-PAMCl is administered orally to rats, the plasma oxime level rises rapidly, reaches a maximum after about 1 hr, and then

---

* Potency ratio: LD50 for 2-PAMCl-treated animals/LD50 for controls (atropine only).

** Medical Research Laboratory. Unpublished data.

decreases at a first-order rate. The biological half-life of 2-PAMCl in the rat is estimated to be 1.4 hr.

2. A prophylactic effect of 2-PAMCl, when used in conjunction with atropine, has been demonstrated in rats poisoned with sarin.

3. The protection afforded by 2-PAMCl against sarin poisoning in rats can be correlated with the oxime level in the plasma at the time of sarin poisoning.

LITERATURE CITED

1. Askew, B. M. Oximes and Atropine in Sarin Poisoning. Brit. J. Pharmacol. 12, 340 (1957).

2. Crook, J. W., Goodman, A. I., Colburn, J. L., Zvirblis, P., Oberst, F. W., and Wills, J. H. Adjunctive Value of Oral Prophylaxis With the Oximes 2-PAM Lactate and 2-PAM Methanesulfonate to Therapeutic Administration of Atropine in Dogs Poisoned by Inhaled Sarin Vapor. J. Pharmacol. Exptl. Therap. 136, 397 (1962).

3. Swintosky, J. V. Illustrations and Pharmaceutical Interpretations of First Order Elimination Rate From the Bloodstream. J. Am. Pharm. Assoc. Sci. Ed. 45, 395 (1956).

4. May, J. R., Zvirblis, P., and Kondritzer, A. A. Technical Requirements and Identification of Pralidoxime Chloride and Its Determination in Biological Material. J. Pharm. Sci. 51, 1508 (1965).

5. Sundwall, A. Plasma Concentration Curves of N-Methylpyridinium-2-aldoxime Methane Sulphonate (P_2S) After Intravenous, Intramuscular and Oral Administration in Man. Biochem. Pharmacol. 5, 225 (1960).

APPENDIXES

Appendix		Page
A.	Tables A-I and A-II.....	15
B.	Figures B-1 and B-2.....	17

APPENDIX A

TABLES

Table A-I. Oxime Concentration in Plasma of Rats Following Oral Administration of 2-PAMCl

Time after admin of 2-PAMCl	Dose, 30 mg/kg		Dose, 60 mg/kg	
	No. of rats	Mean concn ±SE	No. of rats	Mean concn ±SE
hr		µg/ml		µg/ml
0.25	8	2.13 ±0.17	9	3.15 ±0.27
0.5	7	3.09 ±0.39	8	5.93 ±0.45
1.0	8	3.80 ±0.17	9	8.47 ±0.40
1.5	8	3.09 ±0.20	9	7.03 ±0.39
2.0	8	1.99 ±0.21	9	4.89 ±0.40
3.0	8	1.46 ±0.18	9	2.97 ±0.14
4.0	8	0.63 ±0.06	9	2.16 ±0.28
5.0	8	0.38 ±0.07	9	1.32 ±0.13
Biological half-life		1.2 hr		1.5 hr

Table A-II. Effect of 2-PAMCl on the Prophylaxis of Sarin Poisoning in Rats Treated With Atropine (17 mg/kg)

Exp. No.	Dose of 2-PAMCl	Time a/	Plasma oxime b/	LD50 of sarin (19/20 CL) c/	Slope ±SE	Potency ratio d/ (19/20 CL)
	mg/kg	hr	µg/ml	µg/kg		
1	—	—	—	126.8 (111.7 - 144.0)	8.3 ±2.1	—
1	30	1.0	4.0	261.0 (200.2 - 340.1)	3.5 ±0.9	2.00 (1.57 - 2.55)
2	—	—	—	126.1 (107.5 - 147.9)	6.5 ±2.0	—
2	60	1.0	8.1	320.7 (262.9 - 391.2)	5.8 ±1.4	2.46 (2.03 - 2.99)
3	—	—	—	135.8 (121.3 - 152.1)	10.8 ±3.6	—
3	30	2.5	1.7	199.2 (169.2 - 234.3)	8.9 ±2.8	1.53 (1.29 - 1.81)
3	60	2.5	4.1	256.2 (211.3 - 310.6)	6.5 ±1.9	1.97 (1.63 - 2.38)

a/ Time lapse between administration of 2-PAMCl and injection of sarin.

b/ Best estimate of average plasma oxime concentration at time of poisoning, from data in table A-1.

c/ Confidence limits.

d/ Potency ratio is LD50 for 2-PAMCl-treated animals/LD50 for controls (atropine only). 2-PAMCl-treated animals in experiment No. 1, 2, and 3 each compared with combined data for controls in experiment No. 1, 2, and 3, where LD50 was 130.3 µg/kg (120.7 to 140.7 µg/kg) and slope was 8.0 ±1.3.

APPENDIX B

FIGURES

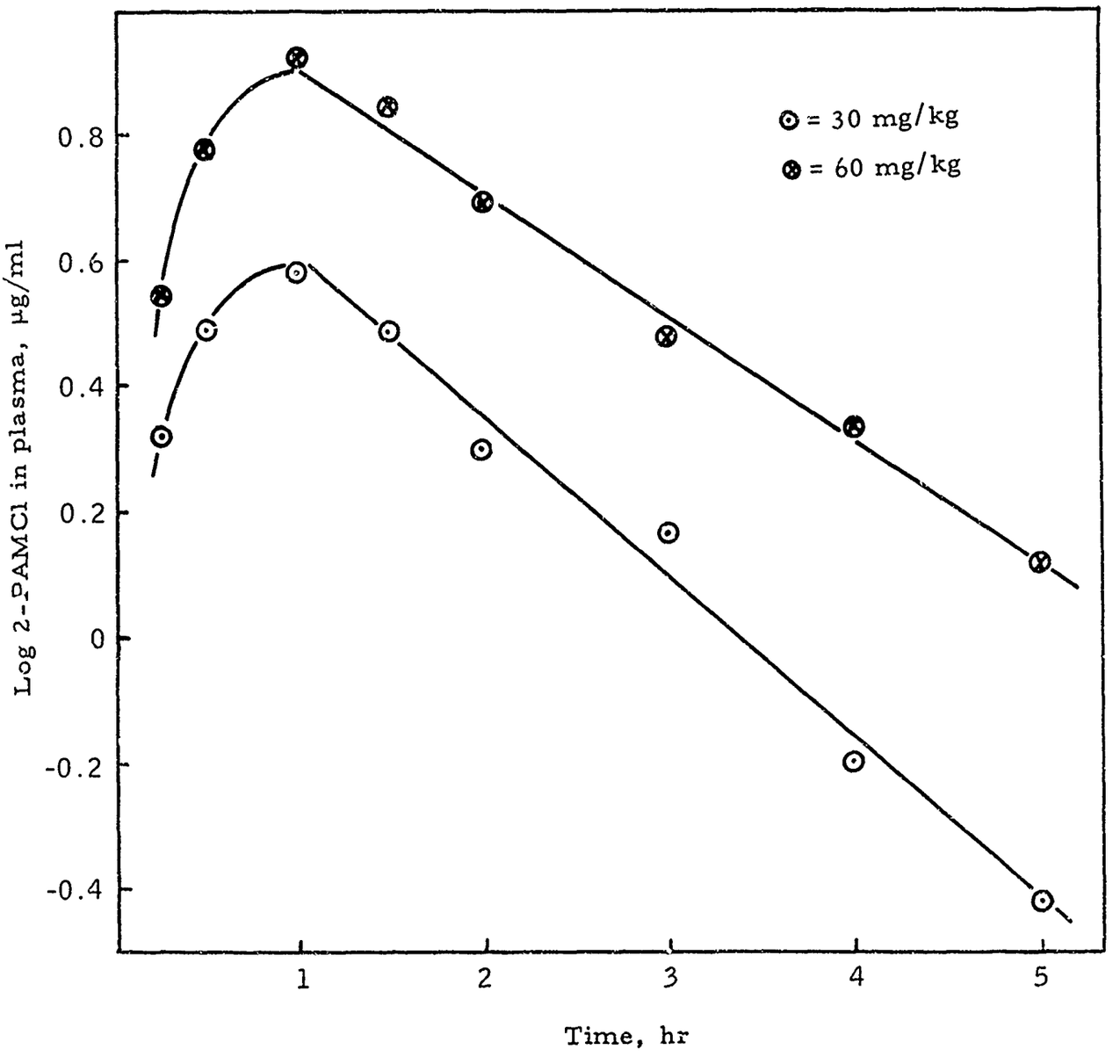

Figure B-1. Concentration of Oxime in Plasma of Rats After Oral Administration of 30- and 60-mg/kg Doses of 2-PAMCl

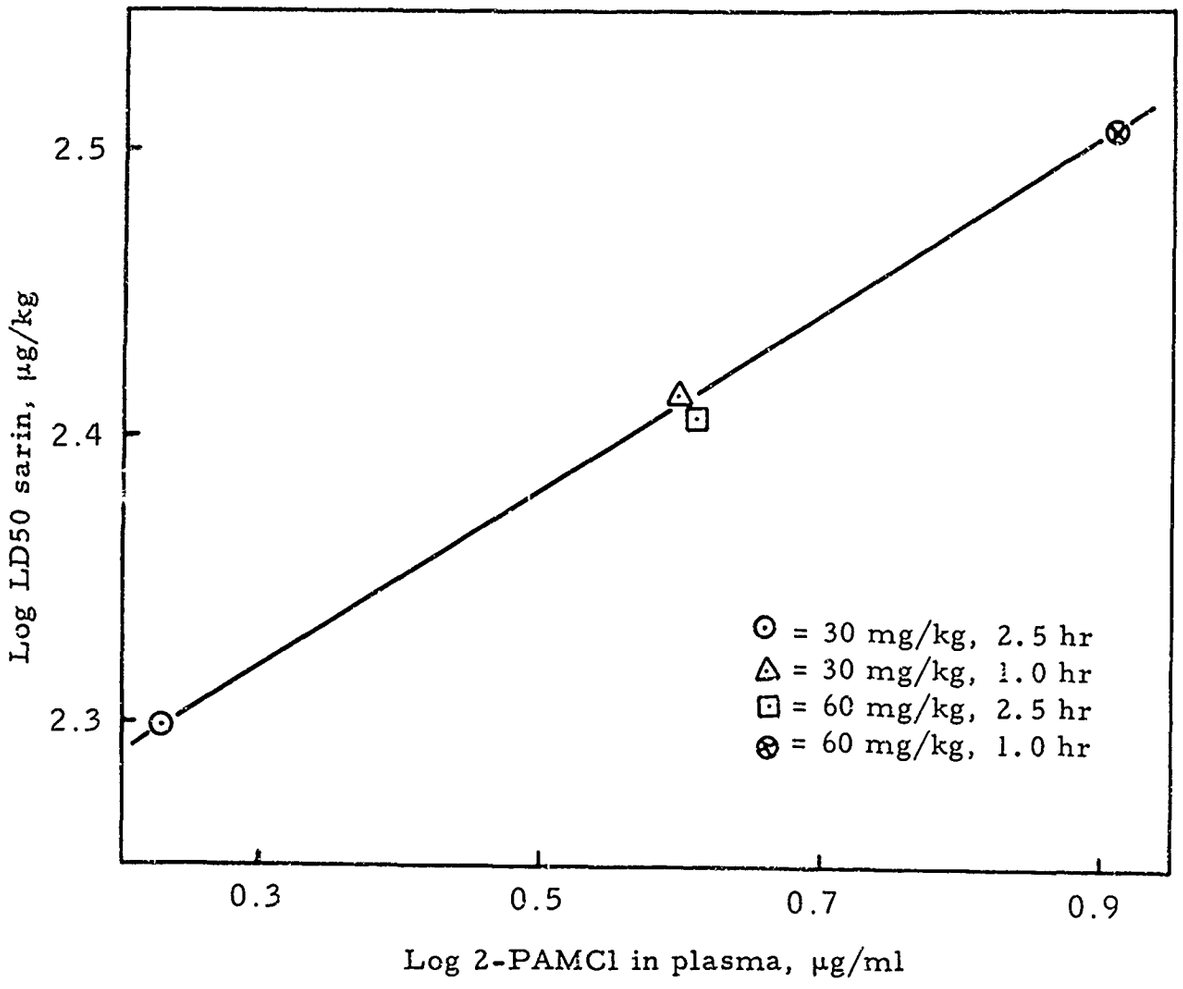

Figure B-2. Effect of Oxime Concentration in Plasma on the Prophylaxis of Sarin Poisoning in Rats

(Animals received 17 mg/kg of atropine intramuscularly following subcutaneous injection of sarin; 2-PAMCl was administered orally as follows: 30 mg/kg 2.5 hr before sarin, 30 mg/kg 1.0 hr before sarin, 60 mg/kg 2.5 hr before sarin, and 60 mg/kg 1.0 hr before sarin)

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) US Army Edgewood Arsenal ATTN:SMUEA-RMP Edgewood Arsenal, Maryland 21010	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP N/A

3. REPORT TITLE

PROPHYLAXIS AGAINST SARIN POISONING IN THE RAT BY ORAL ADMINISTRATION OF 2-PAMCl

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

This work was started in November 1959 and was completed in February 1960.

5. AUTHOR(S) (Last name, first name, initial)

Zvirblis, Peter, and Kondritzer, Albert A.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
November 1966	25	5

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1C622401A097	EATR 4050
c. Task No. 1C622401A09709	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	N/A

10. AVAILABILITY/LIMITATION NOTICES

Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
Non-defense medical aspects of chemical agents	N/A

13. ABSTRACT (U) The aims of the present study were to (a) establish the parameters associated with oxime concentration in plasma following oral administration of 2-PAMCl (2-pyridine aldoxime methochloride) to rats, (b) determine the combined effect of prophylaxis with 2-PAMCl and treatment with atropine on the toxicity of sarin (isopropyl methylphosphonofluoridate, GB) in rats, and (c) establish a correlation between oxime level in plasma and protection afforded against sarin poisoning. It was concluded that: (1) When 2-PAMCl is administered orally to rats, the plasma oxime level rises rapidly, reaches a maximum after about 1 hr, and then decreases at a first-order rate. The biological half-life of 2-PAMCl in the rat is estimated to be 1.4 hr. (2) A prophylactic effect of 2-PAMCl, when used in conjunction with atropine, has been demonstrated in rats poisoned with sarin. (3) The protection afforded by 2-PAMCl against sarin poisoning in rats can be correlated with the oxime level in the plasma at the time of sarin poisoning.

14. KEYWORDS

Prophylaxis	2-PAMCl	LD50
Sarin	Man	Atropine
Rat	Plasma concentration	Biological half-life
Oral administration	Toxicity	